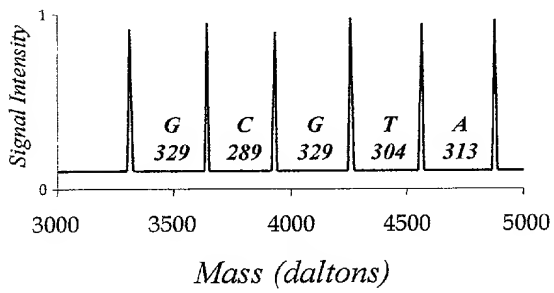
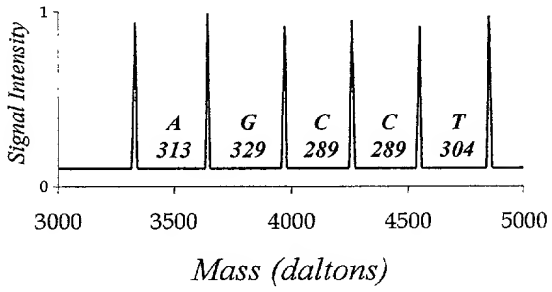
a) *Sequence #1: AGCGTA*

Primer 3016 Da	Extension Products	Mass (Da)
———	agcgta	4878.2
———	agcgt	4565.0
———	agcg	4260.8**
———	agc	3931.6
———	ag	3642.4*
———	a	3313.2

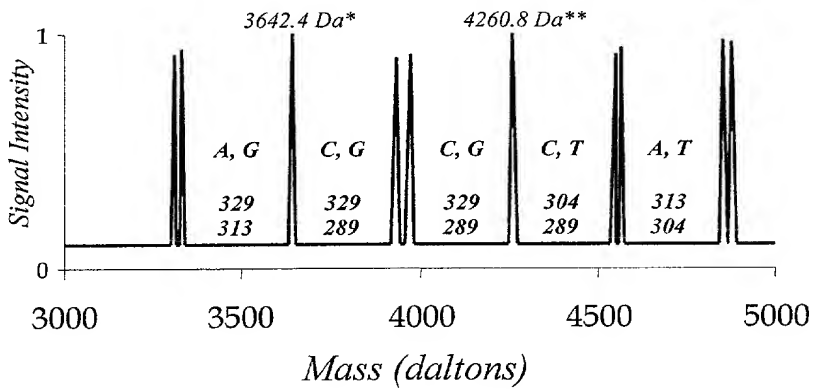
b) *Sequence #2: GATCCT*

Primer 3016 Da	Extension Products	Mass (Da)
———	gagcct	4854.2
———	gagcc	4550.0
———	gagc	4260.8**
———	gag	3971.6
———	ga	3642.4*
———	g	3329.2





c)



d)

e)

gcgct
GCGTA
ggcct
ggcta
acgct
acgta
AGCCT
agcta

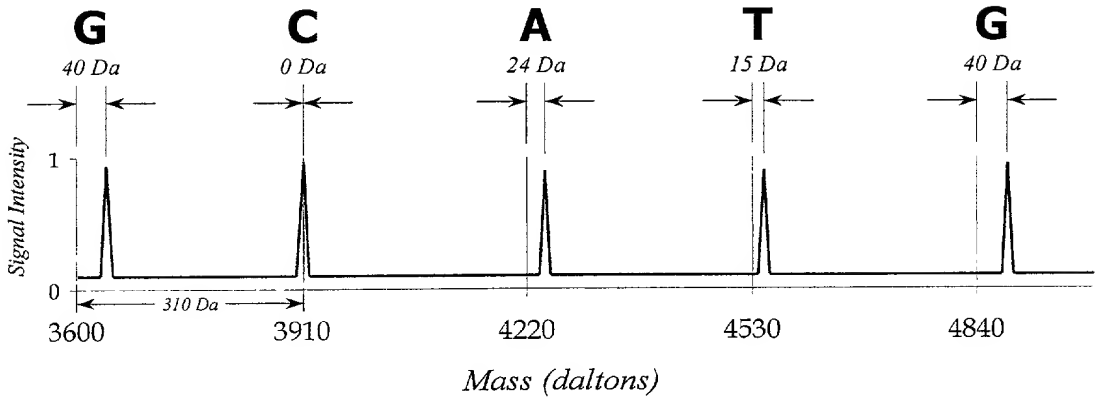
Figure 1

a)

Nucleotide	Mass (Da)
*d*N	310
*dd*C	273
*dd*T	288
*dd*A	297
*dd*G	313

Sequence #1: GCATG

Primer #1 3327 Da — Extension Products	Mass (Da)
nnnng	4880
nnnt	4545
nna	4244
nc	3910
g	3640



b)

Sequence #2: CATGC

Primer #2 3404 Da — Extension Products	Mass (Da)
nnnnc	4917
nnng	4647
nnt	4312
na	4011
c	3677

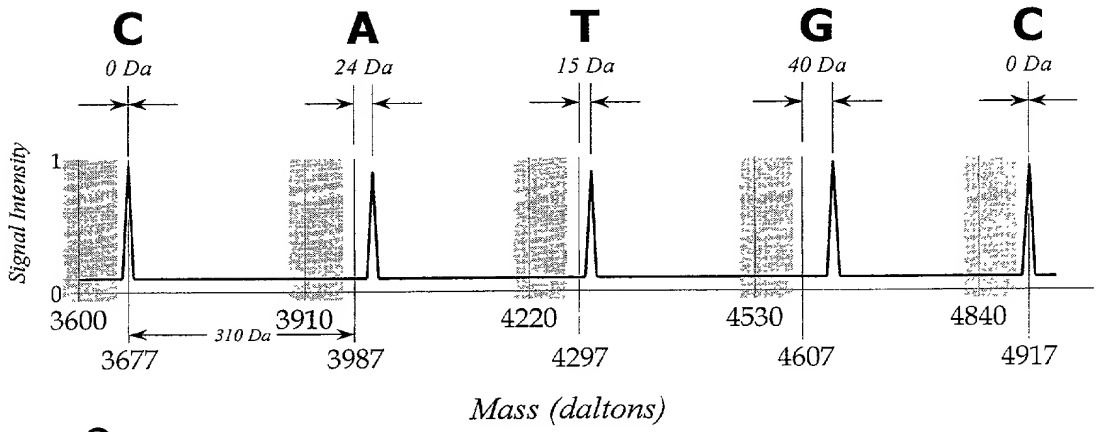


Figure 2

Sequence #1: GCATA

Primer #1 3327 Da	*Extension Products*	*Mass (Da)*
———	nnnna	4864
———	nnnt	4545
———	nna	4244
———	nc	3910
———	g	3640

Sequence #3: CATGC

Primer #3 3404 Da	*Extension Products*	*Mass (Da)*
———	nnnnc	4917
———	nnng	4647
———	nnt	4312
———	na	4011
———	c	3677

Sequence #2: TCAGG

Primer #2 3481 Da	*Extension Products*	*Mass (Da)*
———	nnnng	5034
———	nnng	4724
———	nna	4398
———	nc	4064
———	t	3769

Sequence #4: AACTC

Primer #4 3558 Da	*Extension Products*	*Mass (Da)*
———	nnnnc	5071
———	nnnt	4776
———	nnc	4451
———	na	4165
———	a	3855

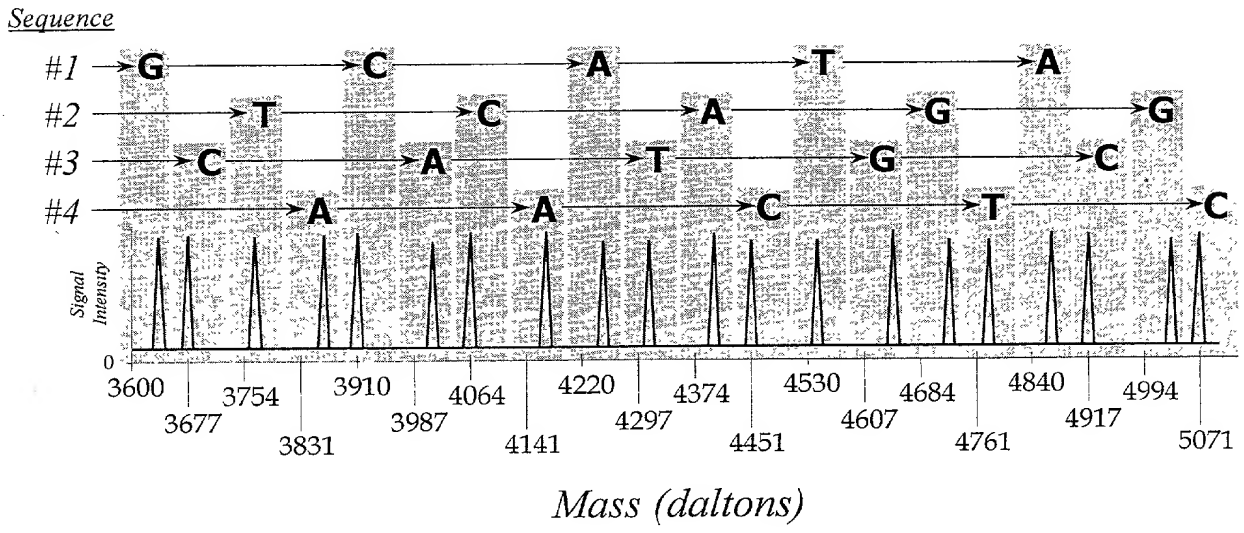


Figure 3

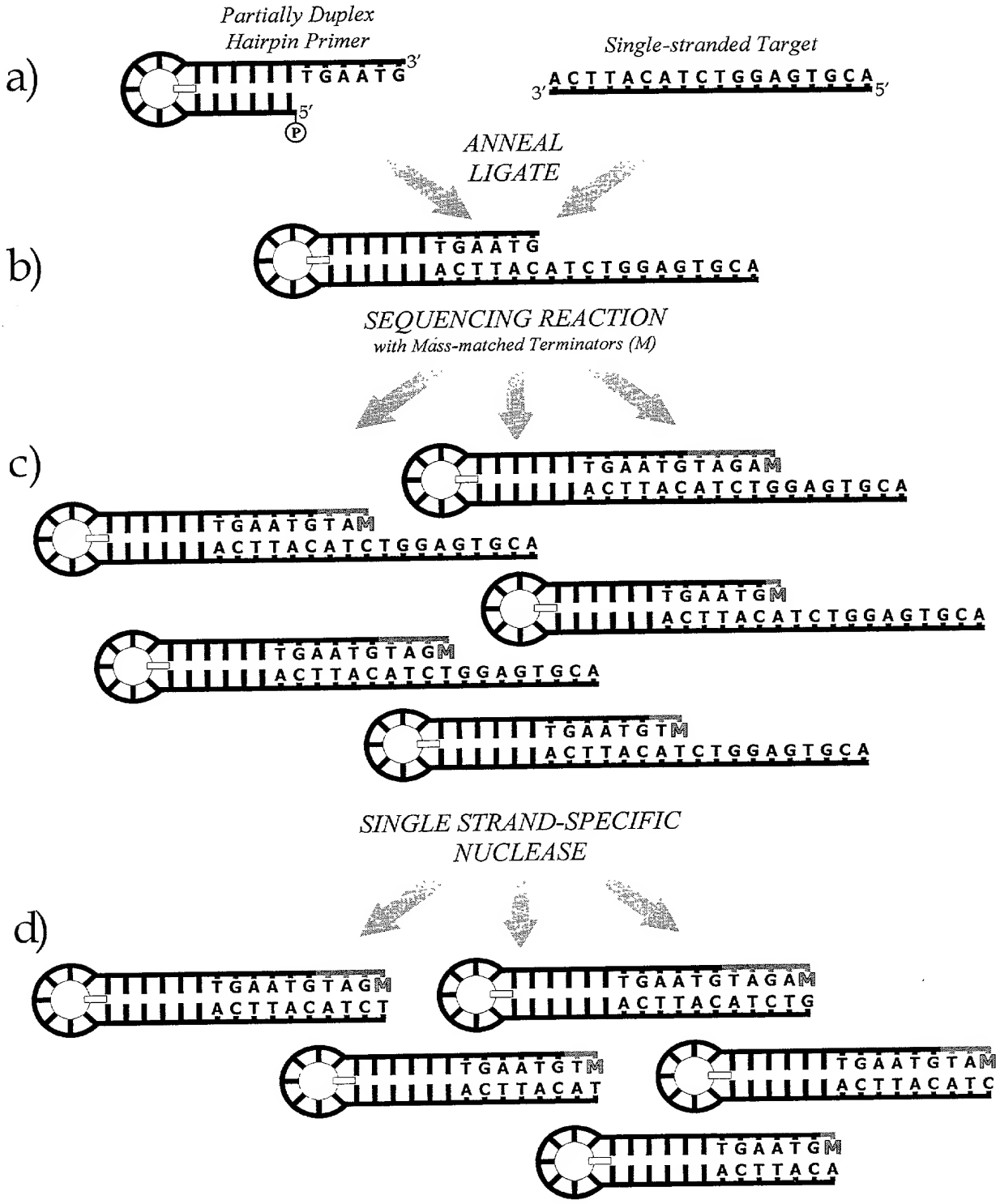

Partially Duplex
Hairpin Primer
Single-stranded Target
a)
TGAATG
3'
5'
P
3'
ACTTACATCTGGAGTGCA
5'
ANNEAL
LIGATE
b)
TGAATG
ACTTACATCTGGAGTGCA
SEQUENCING REACTION
with Mass-matched Terminators (M)
c)
TGAATGTAGAM
ACTTACATCTGGAGTGCA
TGAATGTAM
ACTTACATCTGGAGTGCA
TGAATGM
ACTTACATCTGGAGTGCA
TGAATGTAGM
ACTTACATCTGGAGTGCA
TGAATGTM
ACTTACATCTGGAGTGCA
SINGLE STRAND-SPECIFIC
NUCLEASE
d)
TGAATGTAGM
ACTTACATCT
TGAATGTAGAM
ACTTACATCTG
TGAATGTM
ACTTACAT
TGAATGTAM
ACTTACATC
TGAATGM
ACTTACA

Figure 4

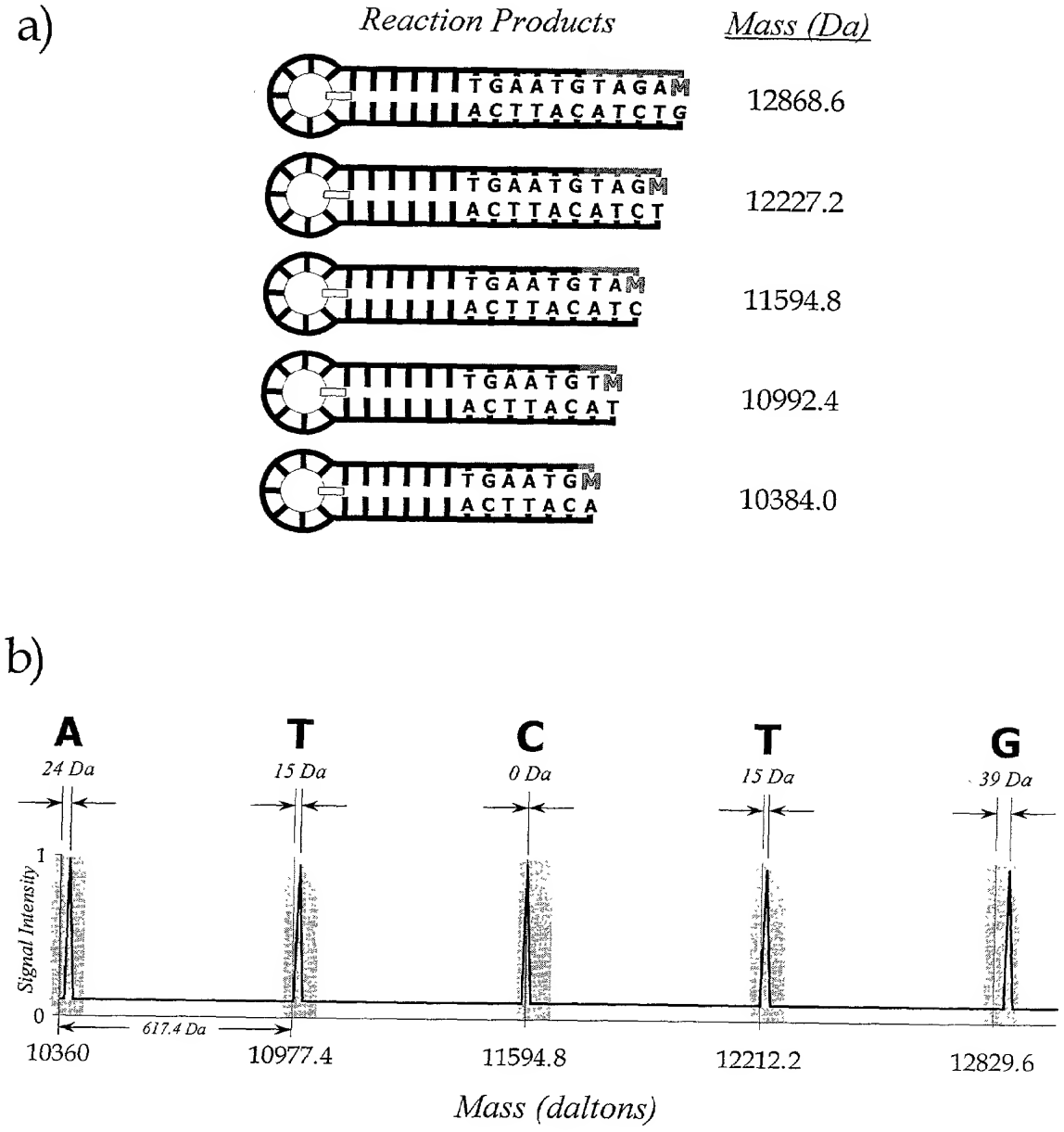
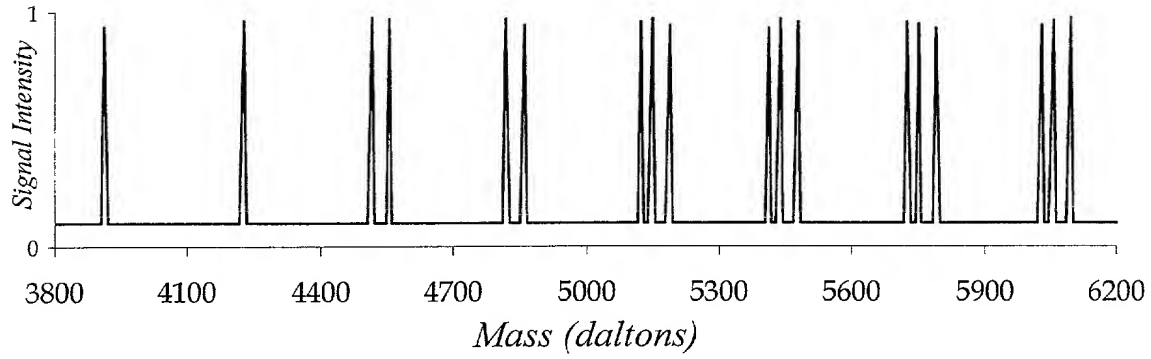

a)
Reaction Products
Mass (Da)
TGAATGTAGAM
ACTTACATCTG
12868.6
TGAATGTAGM
ACTTACATCT
12227.2
TGAATGTAM
ACTTACATC
11594.8
TGAATGTM
ACTTACAT
10992.4
TGAATGM
ACTTACA
10384.0
b)
A
T
C
T
G
24 Da
15 Da
0 Da
15 Da
39 Da
Signal Intensity
1
0
617.4 Da
10360
10977.4
11594.8
12212.2
12829.6
Mass (daltons)

Figure 5

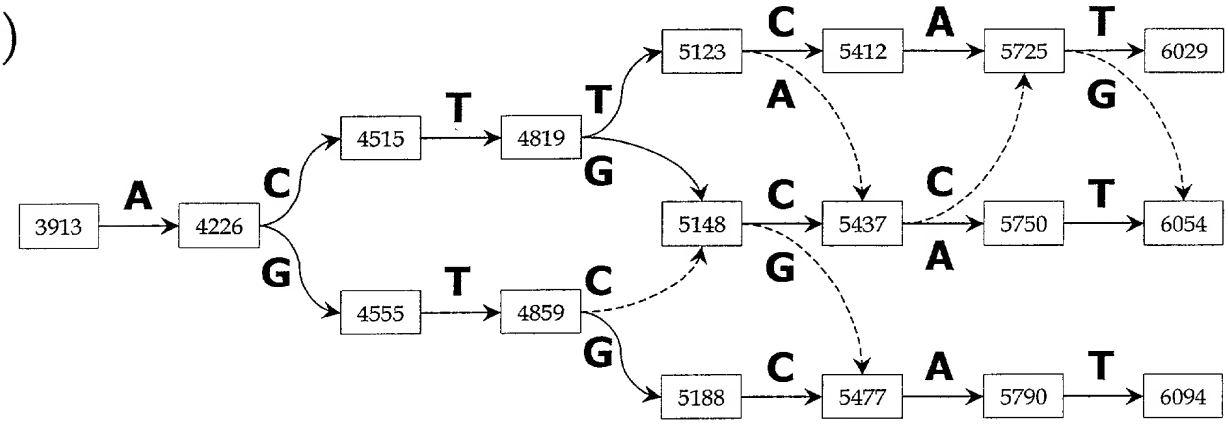
a)

Variant #1: AACTGCAT			Variant #2: AACTTCAT			Variant #3: AAGTGCAT		
Primer 3616 Da	Extension Products	Mass (Da)	Primer 3616 Da	Extension Products	Mass (Da)	Primer 3616 Da	Extension Products	Mass (Da)
———	aactgcat	6054	———	aactccat	6029*	———	aagtgcat	6094**
———	aactgca	5750	———	aactcca	5725*	———	aagtgca	5790**
———	aactgc	5437	———	aactcc	5412*	———	aagtgc	5477**
———	aactg	5148	———	aactc	5123*	———	aagtg	5188**
———	aact	4819	———	aact	4819	———	aagt	4859**
———	aac	4515	———	aac	4515	———	aag	4555**
———	aa	4226	———	aa	4226	———	aa	4226
———	a	3913	———	a	3913	———	a	3913

b)



c)



d)

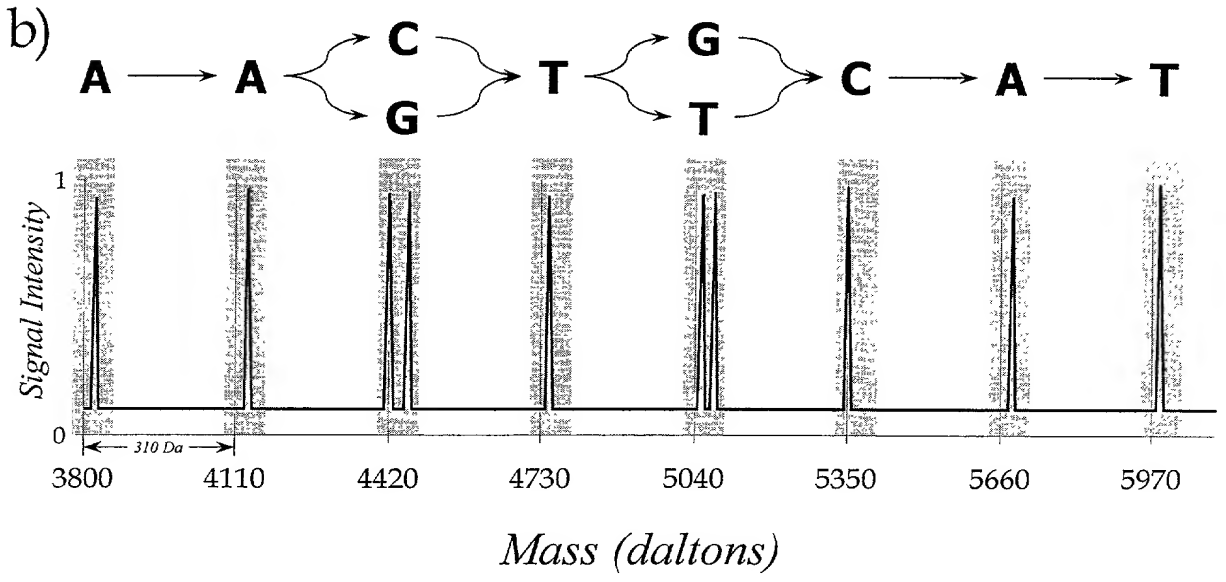
ACTGCAT	actgccg	actgcct	actggat	acttaat	acttacg	acttact
acttcag	ACTTCAT	agtccat	agtcccg	agtccct	agtcgat	AGTGCAT

Figure 6

a)

Variant #1: AACTGCAT			*Variant #2:* AACTTCAT			*Variant #3:* AAGTGCAT		
Primer 3527 Da	*Extension Products*	*Mass (Da)*	*Primer 3527 Da*	*Extension Products*	*Mass (Da)*	*Primer 3527 Da*	*Extension Products*	*Mass (Da)*
	nnnnnnnt	5985		nnnnnnnt	5985		nnnnnnnt	5985
	nnnnnna	5684		nnnnnna	5684		nnnnnna	5684
	nnnnnc	5350		nnnnnc	5350		nnnnnc	5350
	nnnng	5080		nnnnt	5055*		nnnng	5080
	nnnt	4745		nnnt	4745		nnnt	4745
	nnc	4420		nnc	4420		nng	4460**
	na	4134		na	4134		na	4134
	a	3824		a	3824		a	3824

b)



c)

AA(C/G)T(G/T)CAT

d)

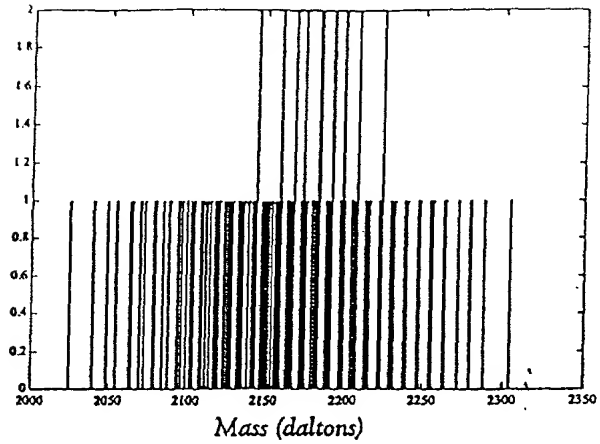
AACTGCAT
AACTTCAT
AAGTGCAT
aagttcat

Figure 7

Figure 8

Base composition density distributions for 7-mers using different nucleotide sets.

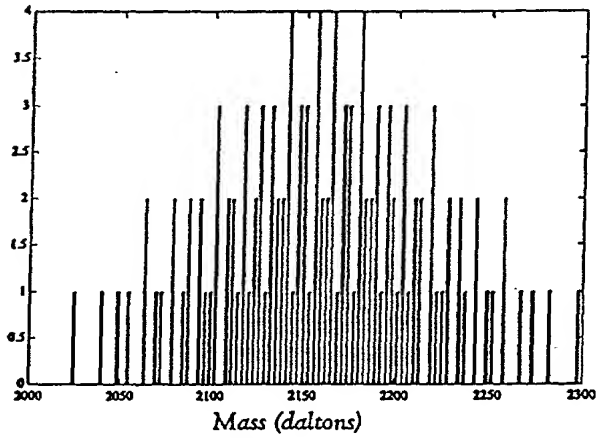
i)



C = 289.2
T = 304.2
A = 313.2
G = 329.2

Naturally Occurring Bases
Peaks can be closer than one dalton.
Total No. of different base compositions = 120
Actual number of represented masses = 110
Avg. No. of compositions per mass value = 1.091

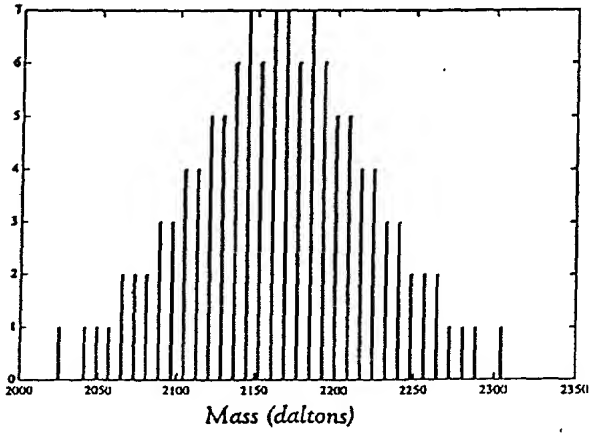
ii)



C = 289.2
T = 304.2
A = 313.2
G = 328.2

Substitution with 7-deaza-dG
Minimum peak separation = 3 daltons
Number of allowed mass values = 92
Actual number of represented masses = 64
Avg. No. of compositions per mass value = 1.875

iii)



C = 289.2
T = 305.2
A = 313.2
G = 329.2

Substitution with deutero-dT
Minimum peak separation = 8 daltons
Number of allowed mass values = 36
Actual number of represented masses = 34
Avg. No. of compositions per mass value = 3.529